

THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of :
Ernest ARENAS et al. : Examiner: Laura L. McGillem
Application No. 09/980,913 :
Filing Date: May 21, 2002 : Group Art Unit: 1636
For: MATERIALS AND METHODS : Atty Docket No: 0380-P02709USO
RELATING TO NEURONAL :
DEVELOPMENT : Confirmation No: 3833

DECLARATION OF PAOLA SACCHETTI PhD

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, PAOLA SACCHETTI, PhD, declare as follows:

1. I am a Postdoctoral research fellow. Details of my appointments, scientific career and publications are given in my *curriculum vitae*, a copy of which is attached as Exhibit A.

2. I am a research fellow in the laboratory of Professor Ernest Arenas who is a co-inventor of the invention claimed in the above-identified patent application (hereinafter "the '913 application").

3. I am a co-author of five (5) publications relating to the transcription factor Nurr1, which are included in my *curriculum vitae* (see Exhibit A).

4. I have been asked to comment on statements made by the Examiner in the final Office Action dated 19 April 2006 and maintained in the Advisory Action dated 13 September 2006, citing Bowen *et al.* US Patent 6284539 ("Bowen") and Takeshima *et al.* (1994) *Neuroscience* 60(3): 809-823 ("Takeshima"). I have been provided with, and reviewed, a copy of these documents, the patent application as originally filed (WO00/66713) and also what I am told

are the claims currently pending before the USPTO. A copy of the claims which I reviewed is attached as Exhibit B.

5. The method for the induction of dopaminergic neurons developed by the inventors, Professor Arenas and colleagues, is a significant achievement in the stem cell field, allowing for induction of high numbers of such neurons.

6. The inventors found that the efficient induction of dopaminergic neurons (for example more than 70% of the total neurons in the culture) can be achieved by the application of two treatments: *Nurr1* overexpression in a stem or progenitor cell, and inductive factors exclusively derived from ventral midbrain astrocytes.

7. The Bowen patent describes a method for generating dopaminergic cells by the introduction and endogenous expression in CNS stem cells of *Nurr1*. Bowen also mentions that co-culturing dopaminergic neurons with striatal astrocytes or with conditioned media from striatal astrocytes or striatal membranes or extracts has been shown to increase the survival of the neurons. See column 3, lines 11-25 of Bowen. However, this was not addressed experimentally in the context of *Nurr1* and was only mentioned as introduction. The idea behind that suggestion is also different from the invention claimed in the '913 application. Bowen proposed *striatal* cells because they are known to be the source of target-derived neurotrophic factors that promote *survival of neurons* and their subsequent morphological differentiation. Thus both the cells suggested by Bowen, (but never used experimentally: striatal cells) and the purpose of that suggestion (neurotrophic support) are different from the cells used and required for use in the Arenas invention (Type 1 astrocytes of the ventral mesencephalon) and the purpose (instruct development of progenitor cells).

8. Bowen achieved poor results. *Nurr 1* was simply transduced into cells and in a typical experiment only 3.5% TH+ cells were obtained out of the total *Nurr1* positive cells by 3 d after transfection" (example 3). This means that even if 100% of the cells would be transfected, the totality of TH+ cells in the culture would be no more than 3.5%. By contrast, the protocol of Professor Arenas and colleagues allows one to obtain 90% TH+ cells out of the total cells in the culture, suggesting that this protocol has virtually complete control over the variables involved in

the process (Nurr1+ all VM astrocyte secreted factors), whereas that by Bowen only takes in account one parameter (nurr1).

9. The examiner correctly states that Takeshima has used astrocytes from the ventral midbrain. However these authors used such cells to treat different cells from those used in the method claimed in the '913 application (neurons instead of progenitors), and for a different purpose. In the Arenas invention, Type 1 astrocytes of the ventral mesencephalon are employed to provide midbrain *inductive factors* to midbrain *progenitors* with the goal of inducing a dopaminergic fate and guiding progenitors in their development until they give rise to newborn dopamine neurons. This is conceptually very different from providing survival promoting factors to already born neurons.

10. It should be noted that the entire protocol by Professor Arenas and colleagues focuses on a developmental time and a developmental process taking place *prior* to the events reported or suggested by either Bowen or Takeshima. Whereas Bowen and Takeshima were concerned with promoting survival of neurons, the protocol of Professor Arenas and colleagues is promoting the development of progenitors into dopaminergic neurons.

11. Takeshima also did not use *Nurr1* in their protocol and did not examine for inductive signals. Takeshima treated cells that were already neuronal cells and then assayed for total numbers of TH+ cells and not for the conversion of progenitors (nurr1+/TH- cells) into dopaminergic neurons (Nurr1+/TH+ cells). The work of Takeshima gives no suggestion of the inductive activity found by Professor Arenas and his colleagues. On the contrary, Takeshima emphasizes the neurotrophic effect of glia.

12. The experiments described in the '913 application and in the inventors' paper Wagner *et al.* (1999) Nature Biotechnology 17, 635 - 636 were not obvious from data in the literature. Neurotrophic factors were expected to act on newborn neurons either in a target derived fashion (hence the experiment suggested by Bowen) or possibly in a paracrine manner (hence the experiment performed by Takeshima). Both experiments focus on maintaining the survival of newborn dopaminergic neurons in a fashion similar to that proposed for glial cell-line derived neurotrophic factor (GDNF). GDNF is first produced in the midbrain, and works in a paracrine manner on dopaminergic neurons (i.e. delivered by neighbor cells in the midbrain).

Later on, as the axons of the DA neurons reach the striatum, GDNF expression in the midbrain is switched off and is turned on in the striatum, to work in a target-derived fashion. Instead, Professor Arenas and colleagues in making the invention proposed that neurotrophic factors are not the critical factor and provided a different approach based on new insight: that progenitors do not develop properly per se *in vitro* and that an instructive factor needs to be supplied to cooperate with *Nurr1*. That idea was new at that time and it is valid still today. There is no suggestion in the disclosures of Bowen and Takeshima, considered individually or together, that a Type 1 astrocyte of the ventral mesencephalon could be used to provide an instructive factor to induce a dopaminergic neuronal fate in a neural stem cell or neural progenitor cell.

I, the undersigned, declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that the statement were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of this application and any patent issuing from this application.

Dated: June 14th, 2007

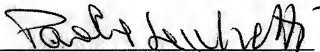
By 
Paola Sacchetti, PhD

Exhibit A

PAOLA SACCHETTI

Laboratory for Molecular Neurobiology
Department of Medical Biochemistry and Biophysics
Karolinska Institutet
Scheeles väg 1, A1 floor 2
SE-171 77 Stockholm
Phone: +46.8.52 48 76 56; Fax: +46.8. 34 19 60; Email: paola.sacchetti@ki.se

Education

2005-now

Postdoctoral Fellow

Karolinska Institutet, Stockholm – Sweden
Mentor: Dr. Ernest Arenas
Research interests: Role of nuclear receptors in ventral midbrain development

2000-2005

Postdoctoral Fellow

INSERM U459, Lille - France
Mentor: Dr. Philippe Lefebvre
Research interests: Physical, functional and pharmacological regulations of the transcriptional activity of the nuclear receptor *nurr1*

2003 -2004 Recipient of a Fondation pour la Recherche Médicale - Foreign
Researcher Fellowship

2001-2003 Recipient of a EU Marie Curie Individual Fellowship

2000-2001 Recipient of a INSERM «Post Vert» Foreign Researcher
Fellowship

2000

Ph.D. in Cellular and Clinical Neurobiology

Wayne State University School of Medicine, Detroit, MI - USA

Mentor: Dr. Michael J. Bannon

Dissertation: Positive and negative elements affecting the transcriptional expression of the human dopamine transporter gene.

2000 Recipient of the Michigan Chapter Award, Society for Neurosciences,
Ann Arbor, MI

1995

Bachelor of Biological Sciences - Wayne State University, Detroit, MI - USA

Research Experience

2005-now

Post-doctoral Research (Karolinska Institutet - Stockholm)
Role of nuclear receptors in ventral midbrain development.

2000-2005

Post-doctoral Research (INSERM U459 - Lille)
Analysis of the transcriptional activity of the nuclear receptor *nurr1* and elucidation of the mechanisms regulating its activity.
Identification of potential pharmacological modulators of the transcriptional activity of *nurr1* and search for potential new dimeric partners.
Other activities included supervising students and a technician and assisting them in experimental design and data analysis.

1995-2000

Graduate Research Assistant (Dept Psychiatry and Behavioral Neurosciences, Wayne State University School of Medicine)
- *Research area*: Characterization of the cis- and trans-elements regulating the

transcriptional expression of the human dopamine transporter gene. Advisor: Michael Bannon, Ph.D.

- *Research area*: Understanding the mechanisms of action of mood stabilizing agents using the differential display technique. Supervisors: Husseini Manji, M.D. and Guan Chen, Ph.D.

- *Research area*: Effects of general protein kinase inhibitors on tolerance to morphine. Supervisor: Alice Young, Ph.D.

1994-1995 *Student Assistant* (Dept Anatomy and Cell Biology, Wayne State University School of Medicine)

- *Research area*: Development of EW in chicken embryos. Supervisor: Joanne Fujii, Ph.D.

Summer 1994 *Microbiology Assistant Volunteer* (Istituto Villa Marelli, Milano, Italy)

Laboratory area: Analysis of Mycobacteria related to Tuberculosis in patients' specimens

Bibliography

Manuscripts:

1. Sousa KM, Hall AC, Steffensen KR, **Sacchetti P**, Hazenberg C, Gustafsson JA, Arenas E. (2007) Liver X receptors regulate neuronal versus glial cell-fate choices in ventral midbrain dopaminergic progenitors. *In preparation*
2. Carpentier R, **Sacchetti P**, Ségard P, Staels B, Lefebvre P. (2007) The glucocorticoid receptor is a coregulator of the orphan receptor nurrl. *Submitted*
3. **Sacchetti P**, Carpentier R, Ségard P, Cren-Olivé C, Lefebvre P. (2006) Multiple signalling pathways regulate the transcriptional activity of the orphan receptor nurrl. *Nucleic Acids Res*, 34: 5515-27.
4. Jouault T, Ibata-Ombetta S, Takeuchi O, Trinlet P, **Sacchetti P**, Lefebvre P, Akira S, and Poulain D. (2003) Candida albicans phospholipomannan is sensed through Toll-like receptors. *J. Infectious Diseases*, 188: 165-72.
5. Riachy R, Vandewalle B, Kerr Conte J, Moerman E, **Sacchetti P**, Lukowiak B, Gmyr V, Bouckenooghe T, Dubois M, Pattou F. (2002) 1,25-Dihydroxyvitamin D(3) Protects RINm5F and Human Islet Cells against Cytokine-Induced Apoptosis: Implication of the Antiapoptotic Protein A20. *Endocrinol.* 143: 4809-4819.
6. **Sacchetti P**, Dwornik H, Formstecher P, Rachez C, Lefebvre P. (2002) Requirements for Heterodimerization between the Orphan Nuclear Receptor Nurrl and Retinoid X Receptors. *J. Biol. Chem.* 277: 35088-96.
7. Bannon MJ, Pruetz B, Manning-Bog AB, Whitty CJ, Michelhaugh SK, **Sacchetti P**, Granneman JG, Mash DC, and Schmidt CJ. (2002) Decreased expression of the transcription factor NURRL1 in dopamine neurons of cocaine abusers. *Proc. Nat. Acad. Science* 99: 6382-6385.
8. Bannon MJ, Michelhaugh SK, Wang J, **Sacchetti P**. (2001) The human dopamine transporter gene: gene organization, transcriptional regulation, and potential involvement in neuropsychiatric disorders. *Eur Neuropsychopharm.* 11: 449-455.
9. **Sacchetti P**, Mitchell TR, Granneman JG, and Bannon MJ. (2001) Nurrl enhances transcription of the

- human dopamine transporter gene through a novel mechanism. *J. Neurochem.* 76: 1565-1572.
10. **Sacchetti P**, Brownschidle LA, Granneman JG, and Bannon MJ. (2000) Homo sapiens dopamine transporter (SLC6A3) gene, 5' flanking region and partial cds. *GenBank sequence submission*, accession number AF115382.
 11. **Sacchetti P**, Brownschidle LA, Granneman JG, and Bannon MJ. (1999) Characterization of the 5'-flanking region of the human dopamine transporter gene. *Molec. Brain Res.* 74: 167-174.
 12. Bannon MJ, **Sacchetti P**, and Granneman JG. (1998) The dopamine transporter: Potential involvement in neuropsychiatric disorders. In: *Psychopharmacology: The Fourth Generation of Progress* 1998 On-Line edition (S.J. Watson, Ed.). <http://www.acnp.org/citations/GN401000016>.

Oral presentations as Invited Speaker:

1. «Characterization of the interaction and transcriptional role of nurr1 and β -Catenin» (2006) 2nd Annual EuroStemCell Consortium, Bellagio - Italy
2. «Nurr1, an essential and unconventional transcriptional regulator acting in dopaminergic neurons» (2004) CCN Dept, Detroit, MI - USA
3. « Mode d'action et rôle du récepteur nucléaire nurr1 dans les cellules dopaminergiques » (2003) Journée des Neurosciences, Lille - France
4. « Nurr1, un recettore nucleare essenziale per le cellule dopaminergiche: come e perchè di una regolazione della trascrizione non convenzionale» (2003) Dept Pharmacological Sciences, Milan - Italy

Posters:

1. **P. Sacchetti**, G. Brundin, L. Conti, E. Cattaneo, and E. Arenas. Nurr1 and β -catenin : Interaction and Role in the Differentiation of Ventral Midbrain Neural Stem Cells into dopaminergic neurons. 2007. EuroStem Consortium, Bellagio, Italy
2. **P. Sacchetti** and E. Arenas. Nurr1 and β -Catenin: interaction and transcriptional role. 2007. EuroSTELLS Consortium, Montpellier, France.
3. P. Lefebvre, R. Carpentier, C. Olivé-Cren, and **P. Sacchetti**. 2006. Regulation of nurr1 transcriptional activity via physical interactions with protein kinases, Keystone Symposium - Nuclear Receptors 2006. Banff, USA.
4. **P. Sacchetti**, P. Ségard, C. Cren-Olivé, R. Carpentier, and P. Lefebvre. 2004. Identification of potential regulators of the orphan nuclear receptor nurr1 transcriptional activity, Society for Neurosciences 34th Annual Meeting. San Diego, CA USA.
5. **P. Sacchetti**, P. Ségard, R. Carpentier, P. Formstecher, and P. Lefebvre. 2003. Screening for potential interacting proteins with the nuclear receptor nurr1, 5th Brain Research Symposium - Of Mice and Men. New Orleans, LO USA.
6. **P. Sacchetti**, P. Ségard, R. Carpentier, and P. Lefebvre. 2003. The Nuclear receptor Nurr1 and its potential cofactors, EMBO Conference – Biology of nuclear receptors. Villefranche-sur-mer, France.
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8. **P. Sacchetti**, H. Dwornik, P. Formstecher, C. Rachez, and P. Lefebvre. 2002. Requirements for heterodimerization between the nuclear receptor Nurr1 and the retinoid x receptor, RXR, Nuclear Receptors 2002. Huddinge, Sweden.
9. M.J. Bannon and **P. Sacchetti**. 2001. Transcriptional regulation of the human dopamine transporter gene, 7th World Congress on Biological Psychiatry. Berlin, Germany.
10. **P. Sacchetti**, J.G. Granneman, and M.J. Bannon. 2000. Transcriptional elements regulating the human dopamine transporter gene, Society for Neurosciences 30th Annual Meeting. New Orleans, LO USA.
11. **P. Sacchetti**, J.G. Granneman, and M.J. Bannon. 2000. Nurr1 as a transcriptional activator of the human dopamine transporter gene, Nuclear Receptors 2000, Keystone Symposium. Steamboat Springs, CO USA.
12. **P. Sacchetti**, L.A. Brownschidle, J.G. Granneman, and M.J. Bannon. 1999. Effects of the nuclear receptor Nurr1 on the expression of the human dopamine transporter gene, Society for Neurosciences - Michigan Chapter. Lansing, MI USA.
13. **P. Sacchetti**, J.G. Granneman, and M.J. Bannon. 1999. Nurr1 as a transcriptional activator of the human dopamine transporter gene, Society for Neurosciences 29th Annual Meeting. Miami Beach, FL USA.
14. **P. Sacchetti**, J.G. Granneman, and M.J. Bannon. 1998. Characterization of the promoter region of the human dopamine transporter gene, Society for Neurosciences 28th Annual Meeting. Los Angeles, CA USA.
- 13.**P. Sacchetti**, and A. Young. 1996. Effects of the protein kinase inhibitor H-7 on the antinociceptive actions of morphine in rats, Society for Neurosciences 26th Annual Meeting. Washington, DC USA.

Exhibit B

Amendments to the Claims:

1. (Previously presented) A method of inducing a dopaminergic neuronal fate in a neural stem cell or neural progenitor cell, the method comprising: expressing *Nurr1* above basal levels within the cell, co-culturing the cell with a Type 1 astrocyte of the ventral mesencephalon, and thereby contacting the cell in vitro with one or more factors secreted from said Type 1 astrocyte of the ventral mesencephalon, whereby dopaminergic neurons are produced.
2. (Previously presented) A method according to claim 1 comprising contacting the cell with fibroblast growth factor 8 (FGF8).
3. (Original) A method according to claim 1 comprising transforming a neural stem cell or neural progenitor cell with *Nurr1*.
4. (Canceled)
5. (Previously presented) A method according to claim 1 wherein the Type 1 astrocyte is immortalized or is of an astrocyte cell line.
6. (Previously presented) A method according to claim 1 wherein said cell is mitotic when it is contacted with said one or more factors.
7. (Previously presented) A method according to claim 1 wherein said cell is additionally contacted with one or more agents selected from the group consisting of: basic fibroblast growth factor (bFGF) epidermal growth factor (EGF), an activator of the retinoid X receptor (RXR), and 9-cis retinol.
8. (Previously presented) A method according to claim 1 wherein said cell is additionally contacted with a member of the fibroblast growth factor (FGF) family of growth factors.
9. (Original) A method according to claim 8 wherein

said cell is contacted with bFGF or EGF, and SR11237.

10. (Previously presented) A method according to claim 1 wherein the neural stem cell or neural progenitor cell is pretreated with bFGF and/or EGF prior to contacting the cell with one or more factors secreted from a Type 1 astrocyte of the ventral mesencephalon.
11. (Previously presented) A method according to claim 1 further comprising formulating a dopaminergic neuron produced by the method into a composition comprising one or more additional components.
12. (Original) A method according to claim 11 wherein the composition comprises a pharmaceutically acceptable excipient.
- 13.-69. (Canceled)